O2 affects the activity of amikacin on mycobacterium abscessus biofilm

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Conclusions: This study suggests that piperacillin-tazobactam therapy in combination with tobramycin in pediatric CF patients may cause a greater incidence of AKI when compared to cefepime.

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LASR REGULATES *PSEUDOMONAS AERUGINOSA*-MEDIATED NEUTROPHIL EXTRACELLULAR TRAP FORMATION

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Introduction:*Pseudomonas aeruginosa* is a major pulmonary pathogen in cystic fibrosis (CF), associated with increased morbidity and mortality. *P. aeruginosa* utilizes different mechanisms to colonize CF airways despite host immune responses. Mutations in the quorum sensing regulatory gene *lasR* confer a survival advantage to *P. aeruginosa* and appear to impart increased pathogenicity in CF patients. The mechanism(s) by which LasR-deficient *P. aeruginosa* evades neutrophil-mediated bactericidal functions is unknown. Neutrophil extracellular trap (NET) formation (NETosis) is an important antimicrobial mechanism. The objective of this study is to understand the role of LasR in *P. aeruginosa*-mediated NETosis.

Methods: Purified human neutrophils from healthy donors were treated for 2h at 37°C with PMA (100nM) or different MOIs (1, 10, and 100) of *P. aeruginosa* strains: wild-type (wt), LasR-deficient (D*lasR*), LasB-deficient (D*lasB)*, PqsR-deficient (D*pqsR)*, RhlR-deficient (D*RhlR)*, LasR/PqsR-deficient (D*lasRDpqsR)*, and LasR/RhlR-deficient (D*lasRDRhlR*). The extracellular release of host DNA was quantified by Sytox Green (5 μM) and fluorescence measurement (485/530nm). The role of LasB and LasA in LasR regulated NETosis was examined using recombinant proteins or by pre-treatment of *P. aeruginosa* wt with elastase inhibitor tropolone (0-1mM). The NADPH oxidase-dependence of NETosis was determined by comparing the inhibition of diphenyleneiodonium chloride (DPI;10 μM) and subsequent treatment with *P. aeruginosa*. Data are represented as percent maximum DNA release of 0.5% TritonX treated PMNs. NETs and distribution of NET proteins were evaluated by immunocytochemistry.

Results: Relative to wt, LasR-deficient *P. aeruginosa* strains were markedly deficient in inducing NETs despite comparable motility and lipopolysaccharide expression levels. This effect of LasR-deficiency on NETosis occurred independently of downstream quorum sensing signaling pathways. Rather, LasR-dependent transcriptional induction of *Pseudomonas* elastase LasB and *Pseudomonas* protease LasA appeared to account for the increased NETosis triggered by wild type, relative to LasR-deficient *P. aeruginosa* strains. Finally, we observed phenotypic differences between NETs stimulated by LasR-sufficient and LasR-deficient *P. aeruginosa* strains.

Conclusions: This study demonstrates, for the first time, that LasR is an essential regulator of *Pseudomonas aeruginosa*-mediated NETosis. This effect is independent of the downstream quorum sensing pathways and results from decreased expression of virulence factors LasB and LasA in the absence of LasR. Together, our findings uncover a novel role for LasR in regulating neutrophil function, which could have important implications in the adaptability of this pathogen to innate immune defenses.

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GENE-SILENCING ANTISENSE OLIGOMERS: A NOVEL THERAPEUTIC APPROACH TO COMBAT MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA* IN VITRO AND IN VIVO

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Cystic fibrosis (CF) is a genetic and progressive disease of significant health concern and is often complicated by chronic infection with the highly virulent, multidrug-resistant *Pseudomonas aeruginosa*. Given the dramatically increasing rate of multidrug resistance among CF patients, urgent and novel approaches to therapeutics are needed. We utilize antisense technology, specifically peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs), designed to inhibit mRNA translation of specific essential genes in *P. aeruginosa* and other gram-negative bacteria. We have previously demonstrated the efficacy of *P. aeruginosa* PPMOs targeted to essential genes, such as *acpP* (acyl carrier protein), *lpxC* (UDP-(3-O-acyl)-N-acetylglucosamine deacetylase), and *rpsJ* (30S ribosomal protein S10), in inhibiting growth and biofilm in vitro. Additionally a single, delayed intranasal treatment was shown to significantly reduce the lung bacterial burden in a murine acute pneumonia model. Here we expand upon these data to show that combinations of PPMOs and clinically relevant antibiotics act synergistically in reducing existing biofilms of *P. aeruginosa*. Furthermore, multiple delayed intranasal treatments with AcpP, LpxC, or RpsJ PPMOs within 6-24 hours post-infection results in significantly improved survival of mice compared to controls. In vivo imaging (IVIS) during these experiments further verified the bactericidal effect of the PPMO treatments. These data suggest that PPMOs alone or in combination with antibiotics represent a novel approach to address the problems associated with rapidly increasing antibiotic resistance in *P. aeruginosa*.

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O_2 AFFECTS THE ACTIVITY OF AMIKACIN ON *MYCOBACTERIUM ABSCESSUS* BIOFILM

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Nontuberculous mycobacteria, notably the multi-resistant *Mycobacterium abscessus* (MABSC), are ubiquitous environmental organisms that frequently cause difficult-to-treat chronic lung infections in cystic fibrosis patients (CF). MABSC infections in CF lungs are difficult to successfully treat, due to their natural resistance towards most clinically available antibiotics. MABSC biofilms have been observed in CF lung sputum where oxygen (O_2) consumption caused by polymorphonuclear leukocyte activity creates anaerobic conditions. Accumulating evidence suggests that the efficacy of several bactericidal antibiotics, such as aminoglycosides, is enhanced by stimulation of pathogens' aerobic respiration and decreased by lack of O_2. Current experiments aim to elucidate the role of cell aggregation (biofilms) and study the bactericidal killing of MABSC by amikacin during aerobic and anaerobic conditions.

Inoculums from 5-day-old cultures of MABSC isolates from CF patients grown in Mueller Hinton broth with 5% Tween 80 (MH) were added to aerobic and anaerobic MH media in 20 mL glass vials to achieve 10^5 cells/mL. Samples were sealed with parafilm or an airtight lid and incubated at 37°C, 150 rpm. Samples with various concentrations (%) of Tween 80 were evaluated by confocal laser scanning microscopy on a Zeiss LSM 880 confocal microscope running Zen 2.1 together as well as by micro-respirometry of O_2. Time-kill curves were generated for amikacin treatment in four-fold dilutions from 2 to 512 mg/mL. The number of bacterial colony forming units (CFU) was determined after 1, 3 and 5 days of incubation.

Conclusion: Bacterial disaggregation increased amikacin efficacy suggesting that aggregation contributes to antibiotic tolerance in a fashion similar to other biofilm infections. Low O_2 consumption in MABSC aggregates creates slow-growing or dormant subpopulations, which are protected against amikacin activity. The bactericidal activity of amikacin may be increased by restoring aerobic conditions in sputum, e.g. through hyperbaric oxygen therapy.

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ROLE OF PARANASAL SINUSES IN EARLY *P. AERUGINOSA* INFECTION IN CF

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Introduction: Chronic *Pseudomonas aeruginosa* (*P. aeruginosa*) lung infections are the major cause of morbidity and mortality in cystic fibrosis (CF) patients. A major purpose of treating patients is to prevent or delay chronic *P. aeruginosa* lung infections. During the initial phase of lung infection, bacteria can be treated with early eradication therapy. Following eradication, lung re-infection can occur due to bacteria with the identical genotype as the previous infection. This may be due to re-colonization from the patient's paranasal sinuses. Although the CFTR defect affects respiratory cells in the upper airways (UA) and lower airways (LA) equally, routine microbiological assessment of the UA is not part of standard care.

Aims: The aims of the study were to evaluate, using non-invasive methods, the microbiological status of the UA of CF patients not chronically infected with *P. aeruginosa*. Genotyping of *P. aeruginosa* strains from the UA and LA were also performed in order to assess if the UA may represent a re-infection source.

Methods: During the period 2014-2017, 40 patients not chronically infected by *P. aeruginosa*, according to the Leeds' definition, were evaluated. We simultaneously sampled the LA and UA: the LA by expectorated sputum or deep throat swab and the UA by nasal lavage, using the Mainz method (Mainz JG, et al. Thorax 2009;64:535-40). *P. aeruginosa* strains of all positive samples were genotyped by BOX-PCR.

Results: A total of 49 nasal lavages and concomitant LA specimens were analyzed from 40 patients (age 2-43 years, median 13 years). During the study period 9 out of 49 (18.4%) nasal lavage and 21 (42.9%) LA were found positive for *P. aeruginosa*. From simultaneously collected positive samples, 7 out of 9 specimens (77.7%) carried identical *P. aeruginosa* genotypes in the UA and LA. *P. aeruginosa* isolates from UA were susceptible to the majority of tested antibiotics: 90% of isolates was susceptible to tobramycin, ciprofloxacin, piperacillin-tazobactam, ticarcillin-clavulanic acid and imipenem, 80% to cefepime, 70% to amikacin, 60% to gentamicin and 50% to levofloxacin. No resistance to colistin, meropenem or ceftazidime was found. Only one *P. aeruginosa* isolate showed a mucoid phenotype.

Conclusions: The presence of identical genotypes in the UA and LA suggests that the UA play a role in the acquisition and persistence of *P. aeruginosa* in CF. Nasal lavage appears to be suitable for non-invasive UA sampling even though microbiological assessment of the UA is not currently considered part of the standard care of CF patients. Since the *P. aeruginosa* strains isolated from the UA had low antibiotic resistance, the efficacy of antibiotic treatment of the paranasal sinuses should be evaluated. In summary, upper airway involvement in CF is undertreated and requires prospective investigation and an interdisciplinary consensus on diagnosis and therapy.

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INFECTION PREVENTION AND CONTROL (IP&C) RECOMMENDATIONS AT CF CENTERS IN THE UNITED STATES

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Objective: We sought to determine if IP&C recommendations made in both the 2003 and 2013 IP&C Guidelines would more likely be included in current IP&C policies than recommendations first made in 2013.

Methods: From 12/2015 to 6/2016, CF care centers' program directors provided researchers with their written IP&C policies for CF. Inpatient and outpatient policies that were institutionally approved or awaiting approval and those created by the CF care teams without ''official'' approval were each reviewed by two researchers for selected recommendations made in the 2003 and/or 2013 Guideline (Table). The percent of policies with specific 2003 and/or 2013 recommendations for inpatient and outpatient settings was determined and compared.

Results: Among the 280 CF care centers, 144 responded; 14 replied they had no written policies, or policies without mention of CF; 130 (46%) provided written IP&C policies. Written policies were provided by 38 (29%) adult, 74 (57%) pediatric and 18 (14%) affiliate centers. The percent of centers with policies that contained specific inpatient and outpatient recommendations is shown (Table). When compared to recommendations made in both 2003 and 2013, new 2013 recommendations were more likely included in policies for inpatient settings (p=0.02) than for outpatient settings (p=0.07). The most common recommendations included 2013 recommendations for mask use by patients and Contact Precautions for all patients in both inpatient and outpatient settings (Bonferroni correction, all p<0.05). A few centers' policies included practices not recommended, i.e., mask use by staff for all patients, or unresolved, i.e., airborne isolation for non-tuberculous mycobacteria (NTM).

Conclusions: Among responding centers, mask use by patients and Contact Precautions were rapidly implemented suggesting high rates of acceptance for the use of personal protective equipment. A few centers' policies included not recommended or unresolved practices, suggesting that local experience may guide IP&C recommendations. Future studies should assess the impact of specific IP&C practices at CF care centers.

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Percentage of CF Care Centers with Selected IP&C Recommendations Currently in Place

	Recommended in CFF IP&C Guideline		N (%) of Centers with Recommendation	
Recommendation	2003	2013	Inpatient	Outpatient
Emphasize hand hygiene by patients	both		94 (72%)	89 (68%)
Emphasize hand hygiene by families	both		65 (50%)	68 (52%)
Avoid socialization among CF patients	both		95 (73%)	68 (52%)
Minimize time in CF clinic waiting room	both		NA	94 (72%)
Case-by-case participation in activities outside hospital room	both		72 (55%)	NA
Masks used by all CF patients	Unresolved	+	111 (85%)	113 (87%)
3 foot rule between CF patients	+	NA	8 (6%)	8 (6%)
6 foot rule between CF patients	NA	+	66 (51%)	68 (52%)
Contact precautions (gown and gloves) used by staff for all CF patients	NA	+	117 (90%)	117 (90%)
Perform pulmonary function tests (PFTs) in exam room or PFT lab with negative pressure, HEPA filtration, or 30 min between CF patients	NA	+	NA	71 (55%)
Masks used by staff for all CF patients	NA	Not recommended	16 (12%)	11 (8%)
Implement airborne isolation for NTM	NA	Unresolved	24 (18%)	22 (17%)

NA=not applicable